Lanthanide(III) Chelates of DTPA-Based Glycoconjugates: Lectin-Mediated Medical Imaging Agents

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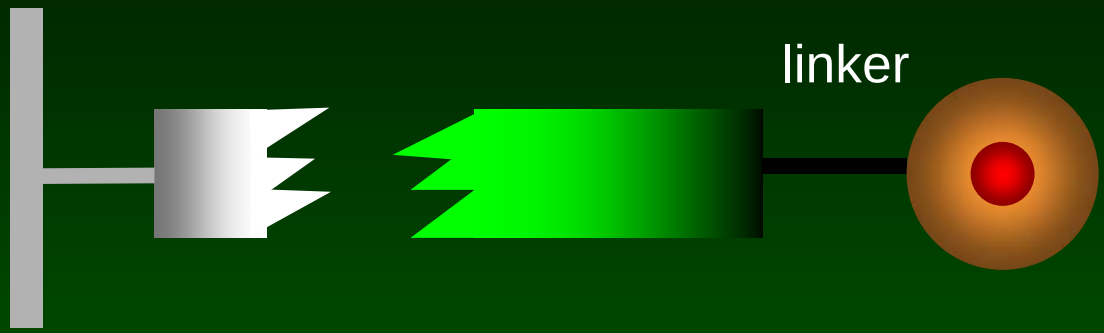
Summary

1. **Objective: Develop new Lanthanide (III) glycoconjugate chelates of DPTA capable of targeting the liver asialoglycoprotein receptor (ASGPR) – lectin with physico-chemical and biological properties capable of making them potential agents for liver imaging**
2. **Synthesis and physico-chemical characterization of dendrimeric DTPA based glycoconjugate ligands**
3. **Physico-chemical characterization of their Ln(III) chelates:**

   - NMR study of the Eu(III) and Sm(III) chelates

   - Relaxivity (NMRD) study of the Gd(III) chelates and their lectin binding; determination of molecular parameters

Active targeting for medical diagnostic



Cell surface receptor (internalization – Intracellular delivery)

Vector molecule (peptide, antibody, carbohydrate,...)

Bifunctional chelator

Metal ion

- Paramagnetic (Gd(III)) : MRI
- Gamma emitter: scintigraphy

Targeting Lectins

Lectins : non-enzymatic proteins or glycoproteins which bind to carbohydrates and act as recognition determinants in many biological processes

- ASGP-R recognizes terminal β-galactosyl residues on desialylated glycoproteins
- Control of intracellular traffic of glycoproteins and liver clearance from the circulatory system of desialylated glycoproteins and apoptotic cells
- Interactions of tumour cells with the immune system, masked by sialyl terminals
- Adhesion of infectious agents to host cells
- Recruitment of leucocytes to inflammatory sites (Selectins)

⇩

Molecular fit between pairs of complementary structures:

lectins ⇔ carbohydrates (ligands)

⇩

This action can be blocked by appropriate sugars *in vivo* and *in vitro*

Liver ASGP Receptor

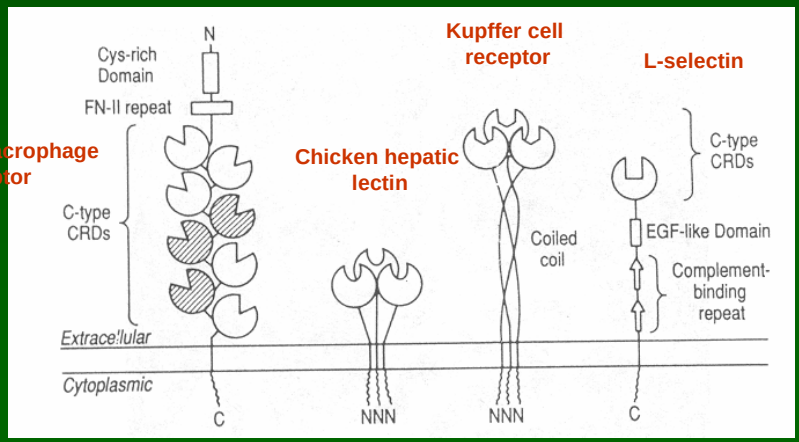
Structural aspects

-Composed of two homologous **subunits**, smaller **H1** and bigger **H2** in humans (ratio 3:1).

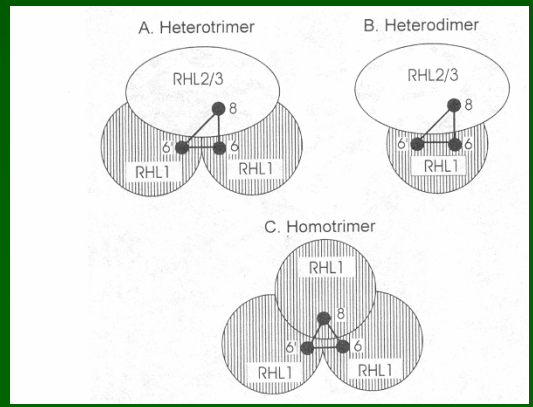
-Each subunit is a type II transmembrane lectin specific to Gal/N-AcGal.

-Each subunit has a short aa cytoplasmatic **N terminus**, a hydrophobic membrane domain, and an exoplasmatic **carboxy terminus** exhibiting a **carbohydrate recognition domain (CRD)** which requires Ca^{2+} ions.

Organization of membrane bound C-type lectins

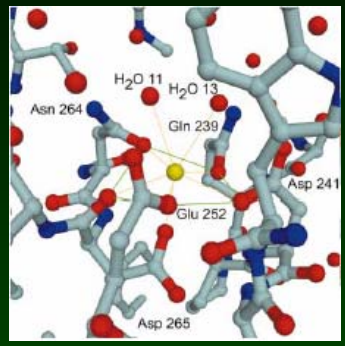


Models of subunit organization of rat ASGP-R (RHL)



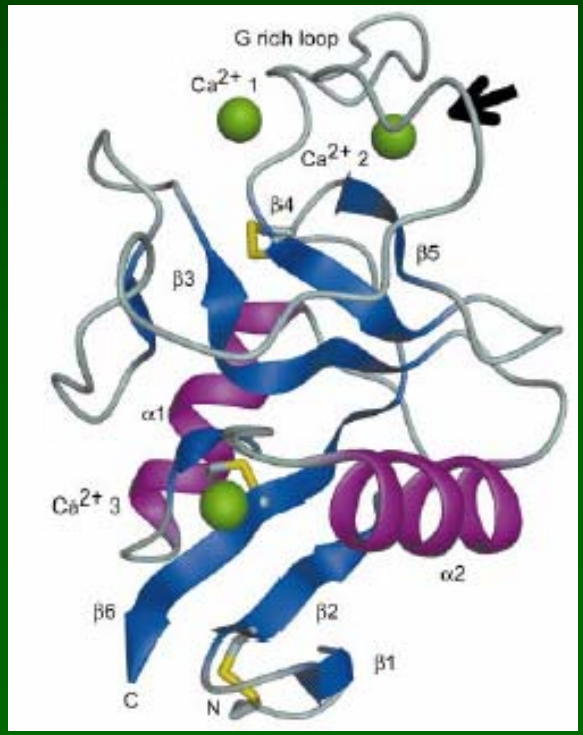
Crystal Structure of CRD of H1 Subunit of ASGP-R

Ca(2) binding site (8 O atoms)

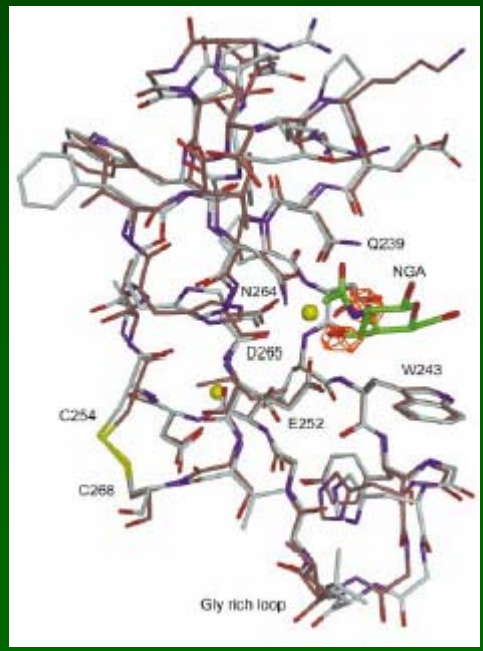


Gal binds at Ca(2) binding site replaces axial water molecules 11 and 13 by (3)OH and (4)OH groups of the carbohydrate

Structure of H1- CRD (aas 147-290)



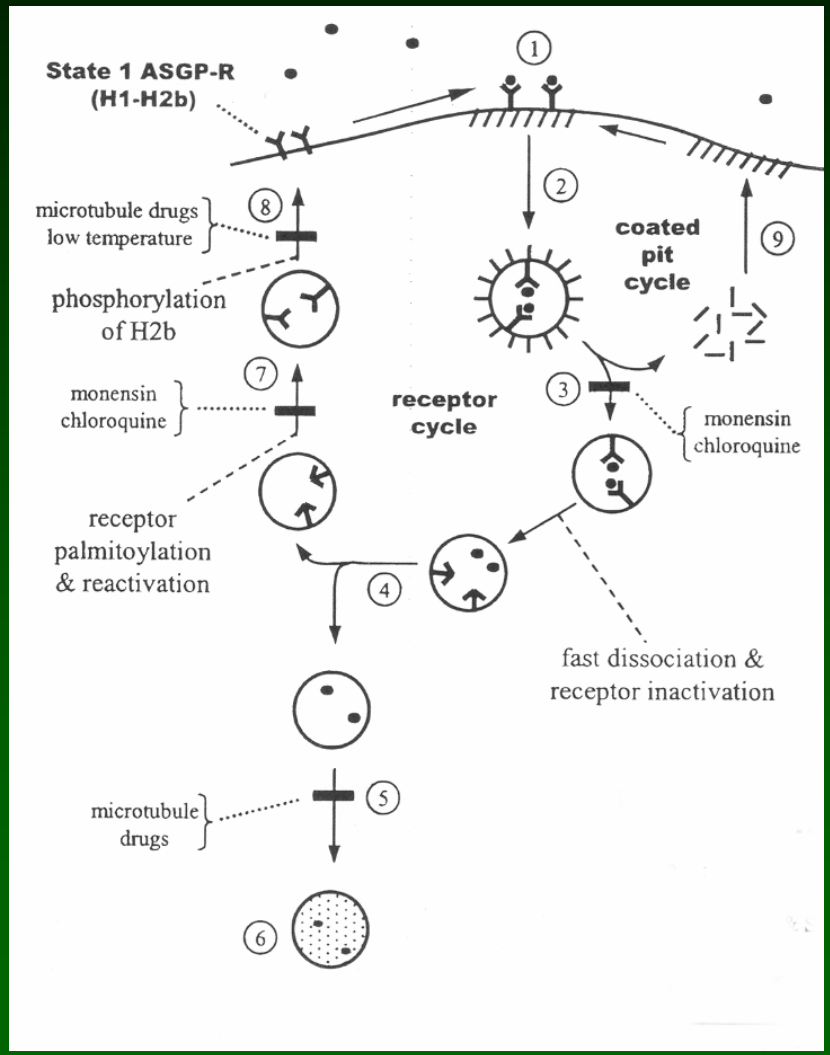
Overlay of sugar binding site of H1-CRD of ASGPR and CDR of MBP mutant containing N-AcGal



Burkhard,…J Mol Biol, 2000

Liver ASGP Receptor

Receptor mediated endocytosis via chlathrin-coated pit pathway



Ligands are cleared from the circulation by receptor-mediated endocytosis and degraded in lysosomes, while the receptor is recycled to the cell surface.

1. Ligand (L) binding
2. Receptor (R)-ligand (L) internalization
3. Uncoating
4. Segregation of R and L
5. L delivery to lysossomes
6. L degradation
7. Reactivation of R
8. Recycling of R to cell surface
9. Formation of new coated pits

Targeting Liver ASGP Receptors

Previous studies

Target ASGP-R: attach galactosyl target residues to a carrier containing efficient reporter groups

reporter - carrier – vector	- technique	- reference
- ^{111}In – DOTA - gal	Gamma Imaging	Meade, et.al., 2003
- ^{99m}Tc - DTPA- GSA (galactosylated HSA)	SPECT	Vera et. al., 2001
- MION - ASF (asialofetuin)	MRI	Brady, Weissleder, et al., 1993
- USPIO – AG (arabinogalactan)	MRI	Brady, Weissleder, et. al., 1991
- SL (spin label)$_n$ – AG	MRI	Gallez, et. al., 1994
- $(GdDTPA)_{858}gal_{2284}$- PL (polylysine – 2136 amino groups)	MRI	Vera, et. al., 1995

Targeting Liver ASGP Receptors

Previous results and our approach

- SPECT quantification of asialoglycoprotein receptor (ASGP-R) correlated with hepatic function in normal vs. pathology
- Tumours (eg. hepatoma) have reduced ASGP-R, uptake of targeted drugs much decreased vs. normal
- ASGP-R present in hepatic carcinoma metastases
- Some of these findings also detected by MRI

Our approach:

- Galactosyl residues were employed as a targeting device as part of dendrimeric glycoconjugates where they were attached to reporter groups containing the Ln(III)-binding moiety, because galactose binding ASGP-R are exposed on the surface of liver parenchimal cells (~500,000/cell)

DTPA- derived dendrimeric glycoconjugates

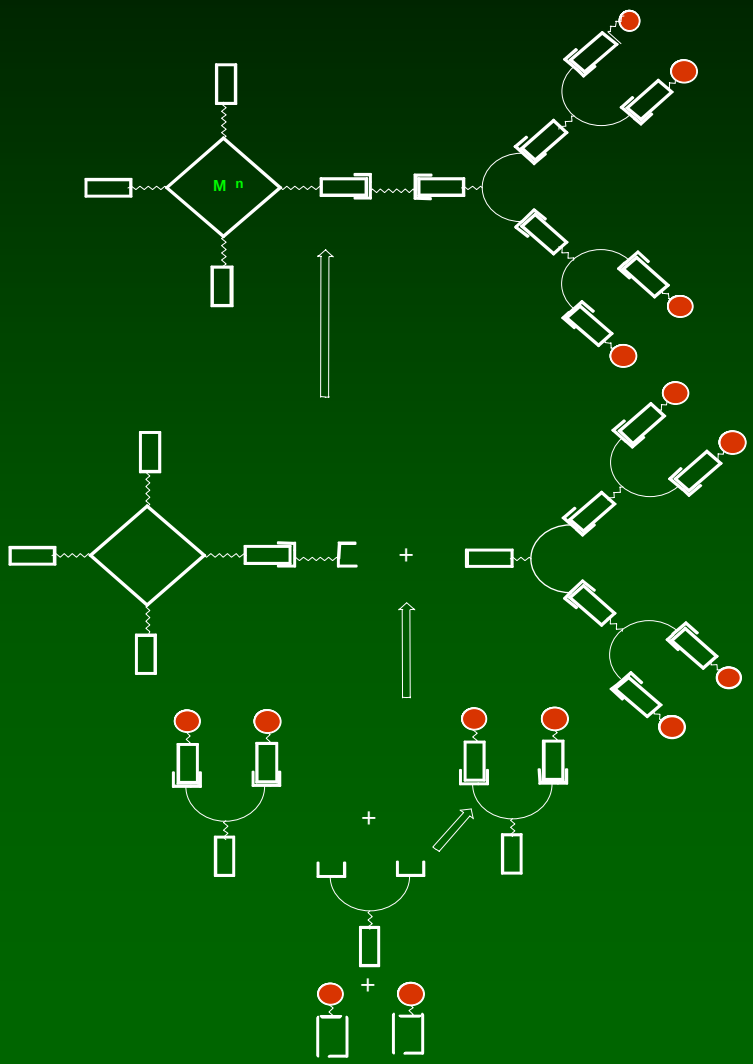
Synthetic Approach

Convergent synthesis

sugar moiety

carboxylic acid group

amine group



Pro-chelator: DTPA bisanhydride

Sugar block: carboxylate functionalized glycodendromer (G0, G1, G2)

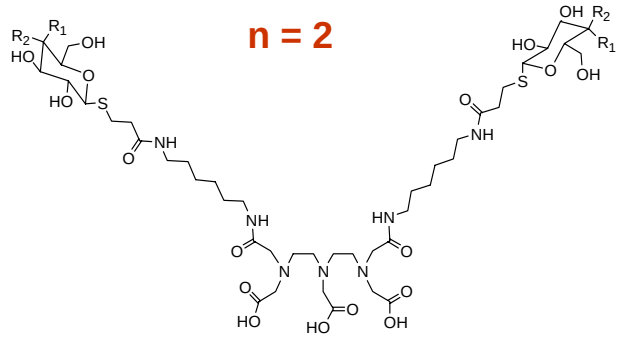
DTPA-bisamide glycoconjugates

Dendrimeric Ln(DTPA-bisamide) glycoconjugates

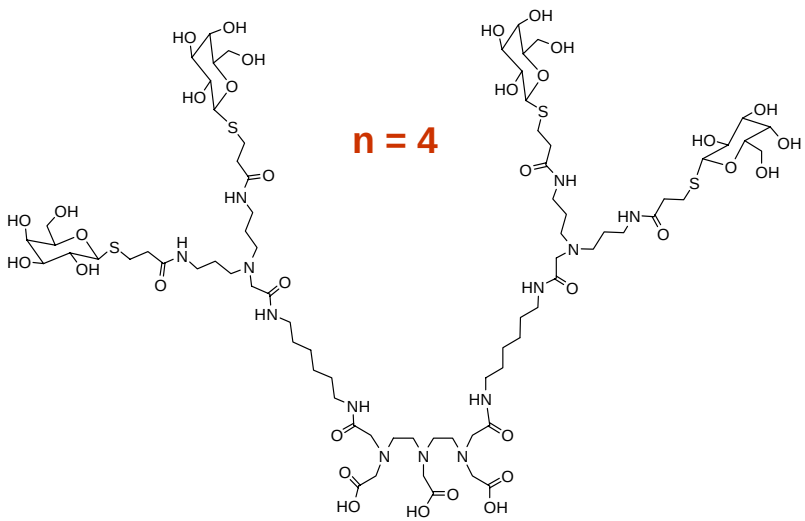
Ln(DTPA)- $(Sugar)_n$

Sugar = Gal, Glc, Lac

n = 2, 4



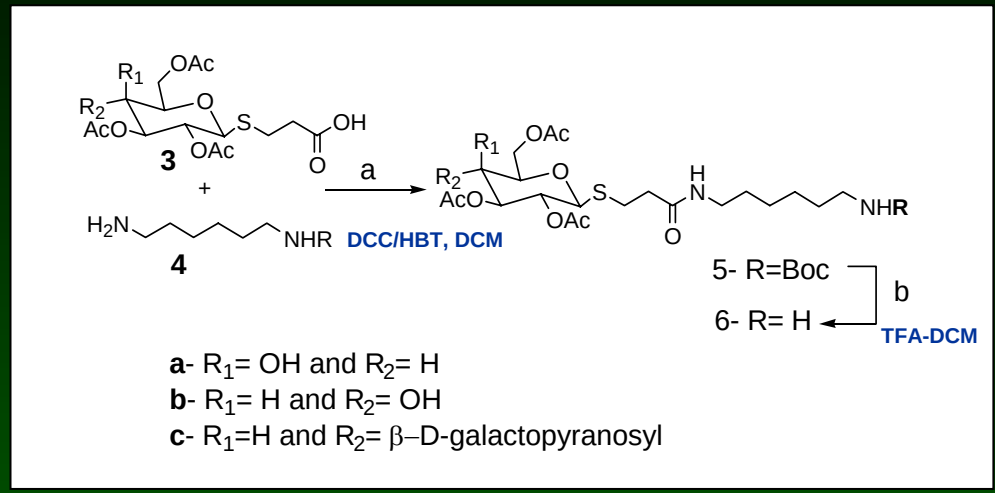
1a- R_1= OH and R_2= H - **$DTPA(Gal)_2$**
1b- R_1= H and R_2= OH - **$DTPA(Glc)_2$**
1c- R_1=H and R_2= β–D-galactopyranosyl - **$DTPA(Lac)_2$**



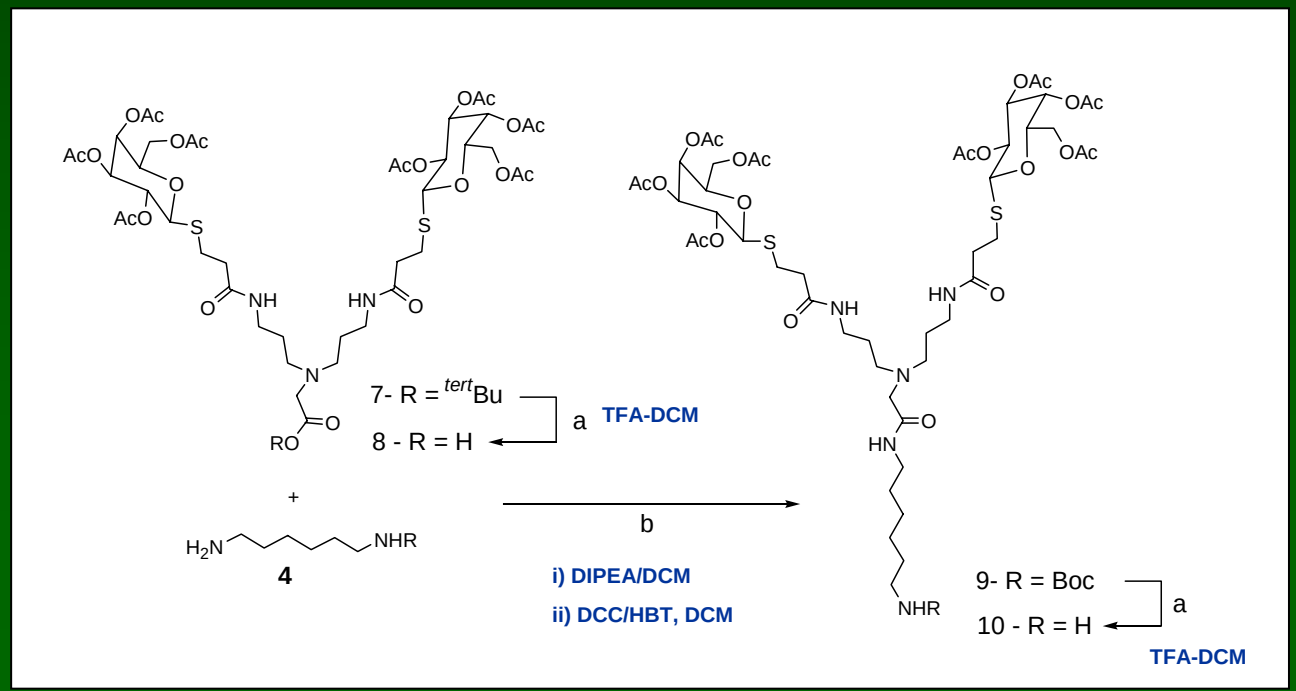
2-$DTPA(Gal)_4$

Synthesis of DTPA-bisamide glycoconjugates

Boc protected HMD-functionalized monovalent thioglycosides and deprotection

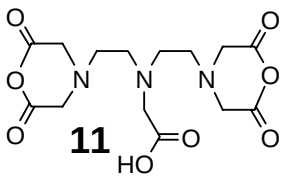


Boc protected HMD-functionalized divalent thioglycosides and deprotection



Synthesis of DTPA-bisamide glycoconjugates

DTPA-bisamide glycoconjugates: mono and divalent dendromers



+ **6** $\xrightarrow{a,b}$ **1**

a- R_1= OH and R_2= H - **DTPA(Gal)$_2$**
b- R_1= H and R_2= OH - **DTPA(Glc)$_2$**
c- R_1=H and R_2= β–D-galactopyranosyl - **DTPA(Lac)$_2$**

11 + 10 $\xrightarrow{a,\ b}$ **2** - **DTPA(Gal)$_4$**

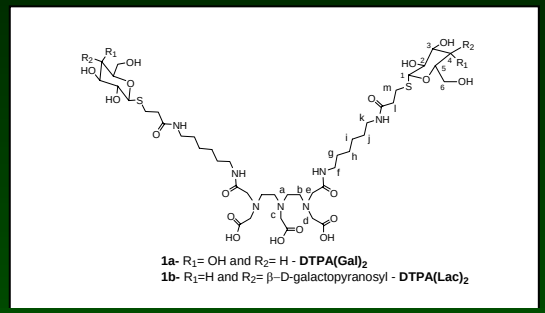
a) i) DIPEA/DCM; ii) DMF

b) i) KOMe/EtOH; ii) Amberlist 15, NH_3(aq)

iii) RPC8 silica, elution with H_2O/MeOH

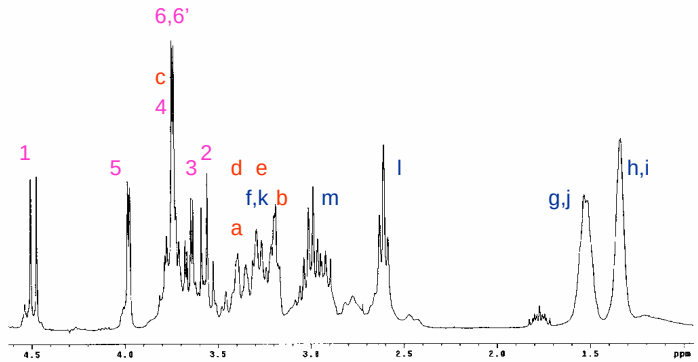
NMR characterization of Ln-DTPA-bisamides chelates

L = DTPAGal$_2$



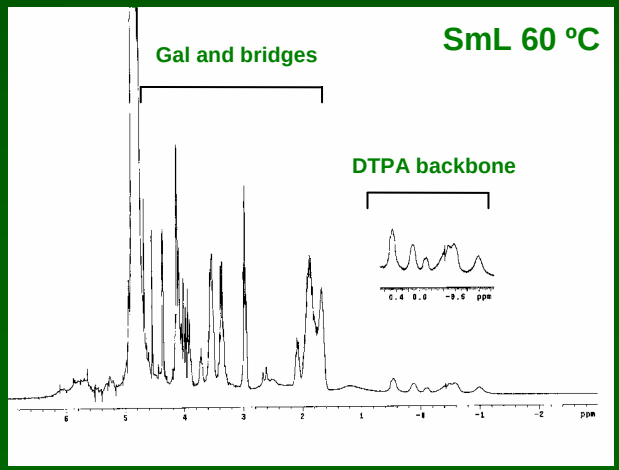
1a- R_1= OH and R_2= H - **DTPA(Gal)$_2$**
1b- R_1=H and R_2= β–D-galactopyranosyl - **DTPA(Lac)$_2$**

LaL 25 ºC

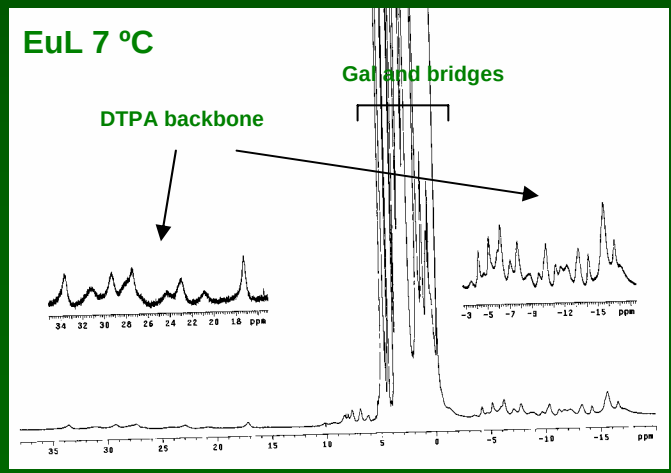


4 diastereoisomeric pairs of enantiomers

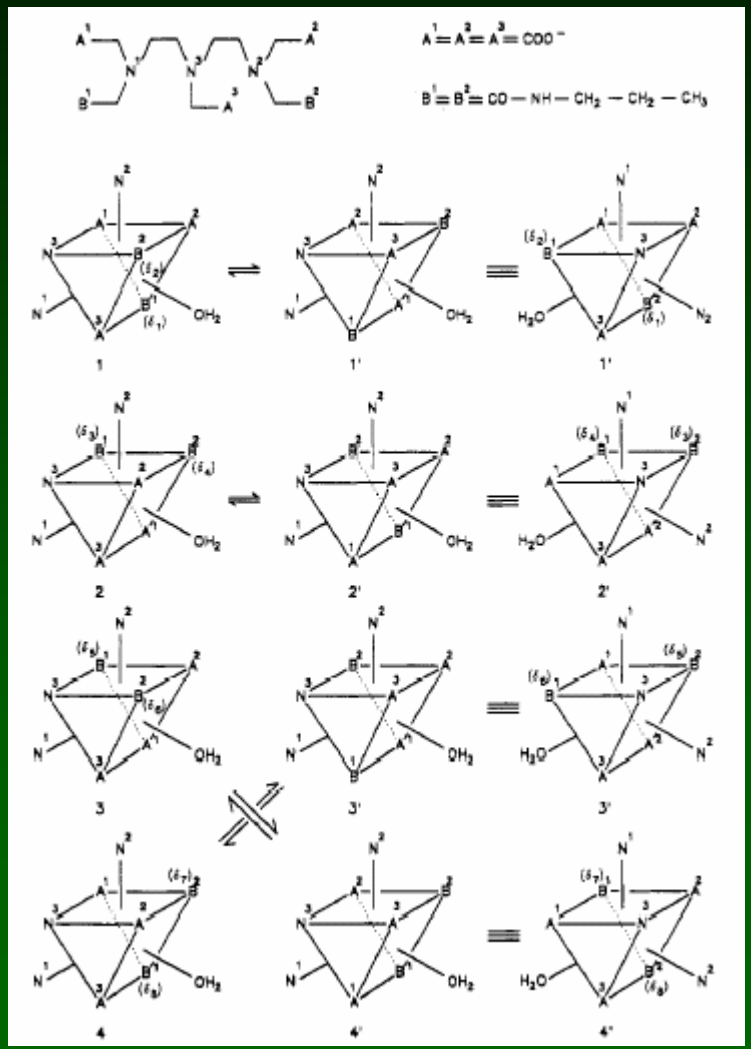
SmL 60 ºC



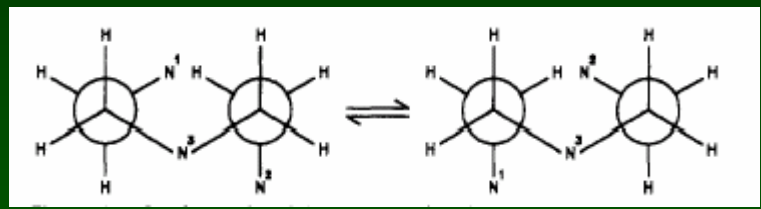
EuL 7 ºC



Coordination polyhedrons of the eight enantiomers of Ln-DTPA-bisamides chelates



Conformational interconversion in the diethylenetriamine backbone of Ln-DTPA-bisamides chelates



Geraldes et al, Inorg. Chem., 1993

SBM Theory of Inner-Sphere Proton Relaxivity

$$r_1 = r_{1is} + r_{1os}$$

Inner- and outer-sphere contributions

$$r_{1is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^{H} + \tau_m}$$

Inner-sphere term exchange – parameters: q, τ_M

Inner-sphere T_{1M} parameters:

$$\frac{1}{T_{1m}{}^{H}} = \frac{2}{15}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\hbar^2 \gamma_S^2 \gamma_I^2}{r_{GdH}^6} S(S+1)\left[\frac{3\tau_{d1H}}{1+\omega_I^2\tau_{d1H}^2} + \frac{7\tau_{d2H}}{1+\omega_S^2\tau_{d2H}^2}\right]$$

r_{GdH}, τ_M, τ_R

$$\frac{1}{\tau_{diH}} = \frac{1}{\tau_m} + \frac{1}{\tau_R} + \frac{1}{T_{ie}}$$

Δ^2, τ_V

T_{1e}, T_{2e}

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_v\left\{4S(S+1)-3\right\}\left(\frac{1}{1+\omega_S^2\tau_v^2} + \frac{4}{1+4\omega_S^2\tau_v^2}\right)$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2\tau_v\left[\frac{5.26}{1+0.372\omega_S^2\tau_v^2} + \frac{7.18}{1+1.24\omega_S\tau_v}\right]$$

Freed Theory of Outer-Sphere Proton Relaxivity

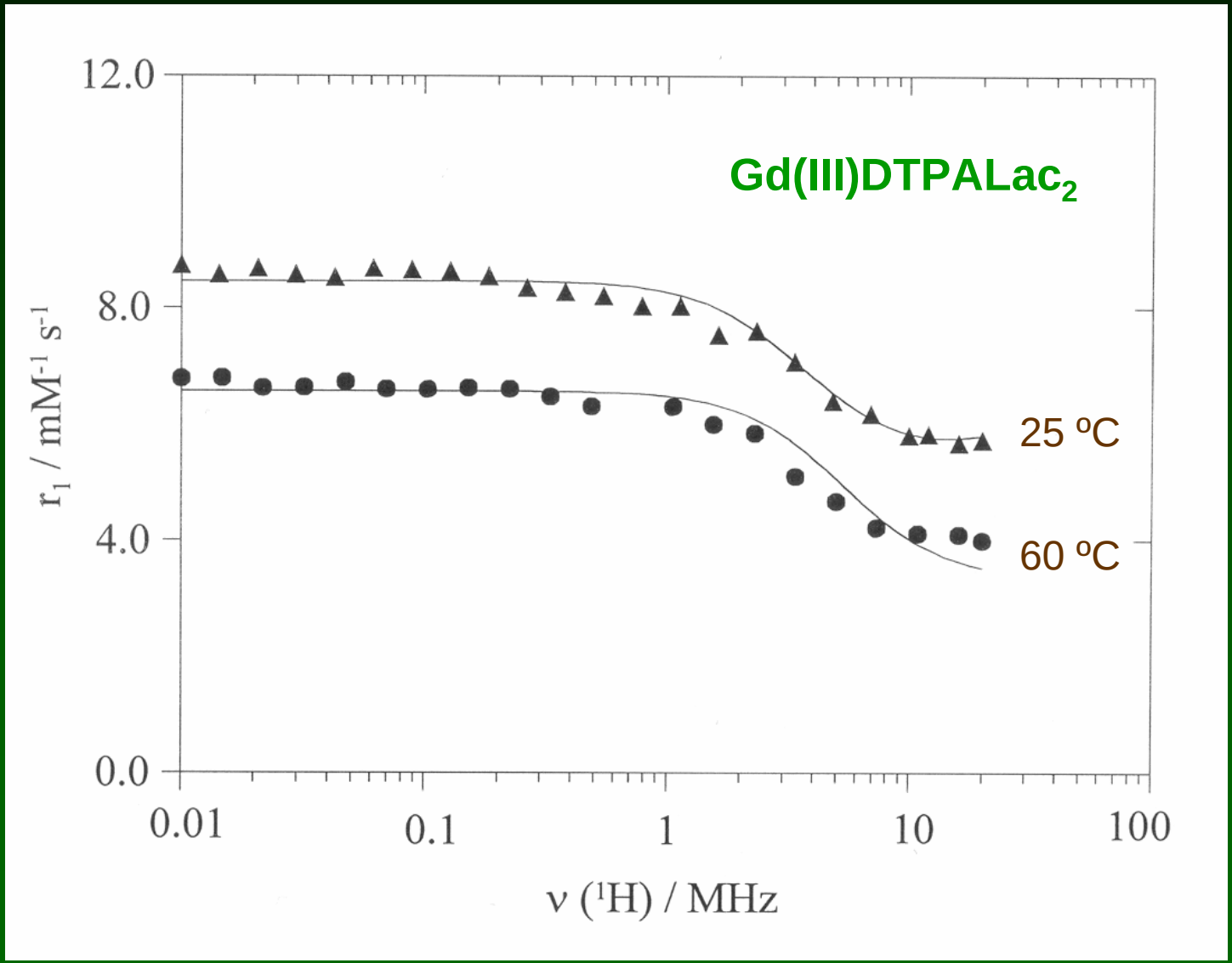
Outer-sphere term – parameters: a_{GdH}, D_{GdH}, Δ^2, τ_v

$$r_{1os} = \frac{32 N_A \pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2 \gamma_S^2 \gamma_I^2}{a_{GdH} D_{GdH}} S(S+1)\left[3J_{os}(\omega_I, T_{1e}) + 7J_{os}(\omega_S, T_{2e})\right]$$

$$J_{os}(\omega, T_{je}) = \mathrm{Re}\left[\frac{1 + \frac{1}{4}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{1/2}}{1 + \left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{1/2} + \frac{4}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right) + \frac{1}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{3/2}}\right]$$

Free diffusion model

NMRD of Gd-DTPA - glycoconjugate



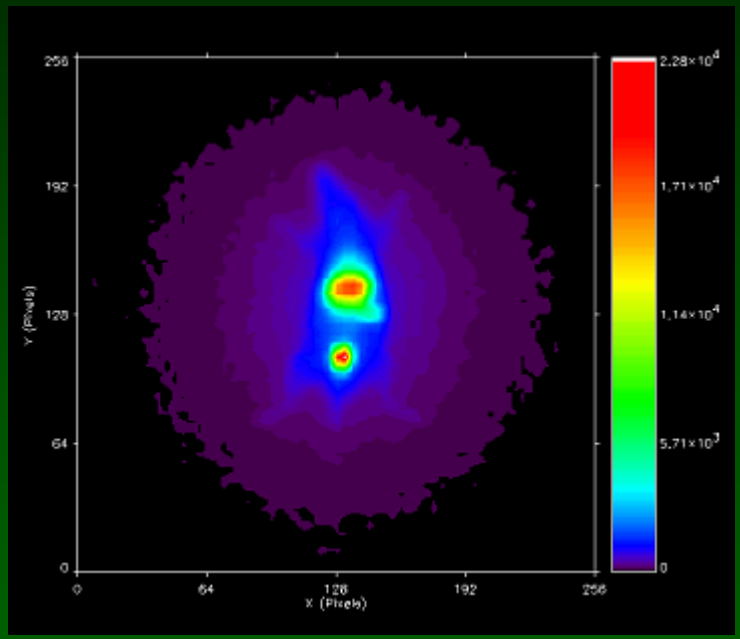
Parameters from the analysis of the NMRD profiles

Gd(III)-DTPA-glycoconjugates

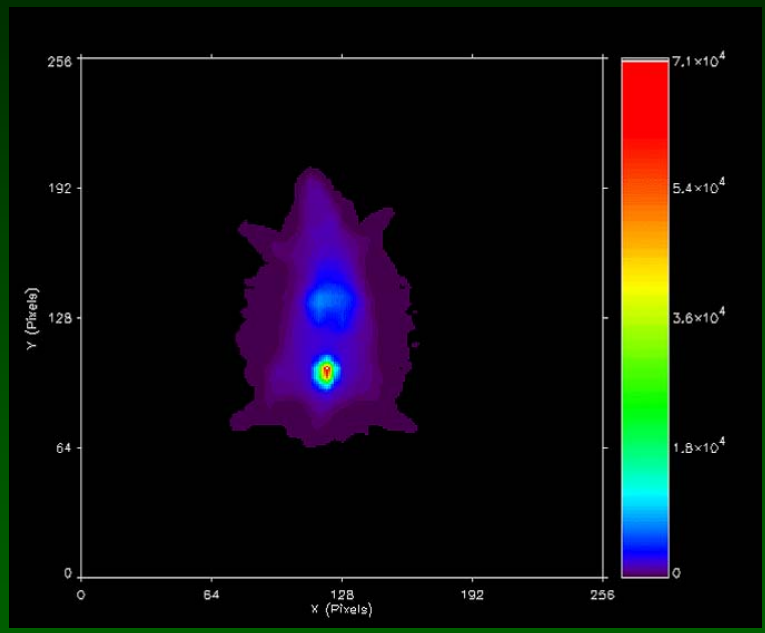
	DTPA	DTPA-BMA	$DTPALac_2$
k_{ex}^{298}/ 10^6 s^{-1}	3.3±0.2	0.45±0.1	0.40
$\Delta H^{\ddagger}$ / kJ mol^{-1}	51.6±1.4	47.6±1.4	40.0
τ_{rH}^{298} / ps	58±11	66±11	332±10
E_{RH} / kJ mol^{-1}	17.3±0.8	21.9±0.5	36.3±0.2
τ_v^{298} / ps	25±1	25±1	10±2
E_v / kJ mol^{-1}[g]	1.6±1.8	3.9±1.4	1
Δ^2 / 10^{20} s^{-2}	0.46±0.02	0.41±0.02	0.63±0.02
D_{GdH}^{298}/ 10^{-10} m^2 s^{-1}	20±3	23±2	24.0
E_{DGdH} /kJ mol^{-1}	19.4±1.8	12.9±2.1	20

green parameters are fixed in the fit; **Gd-DTPA-BENGALAA:** τ_{rH}^{298} = 265 ± 22 ps, Lammers, et al, 1997)

Gamma Scintigraphy of Wistar rats
^{153}Sm(III) labeled glycoconjugates



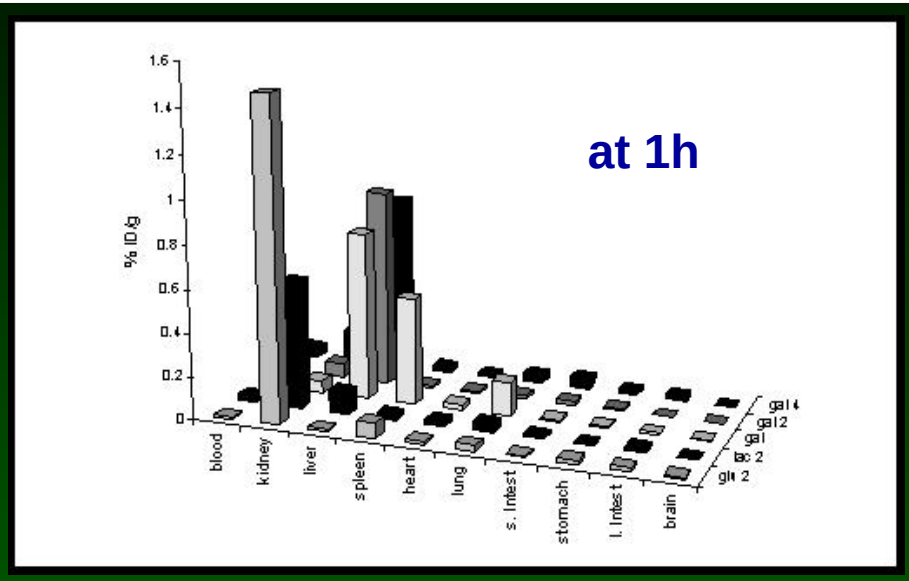
DOTAGal$_2$ 30 min



DOTAGlc$_2$ 30 min

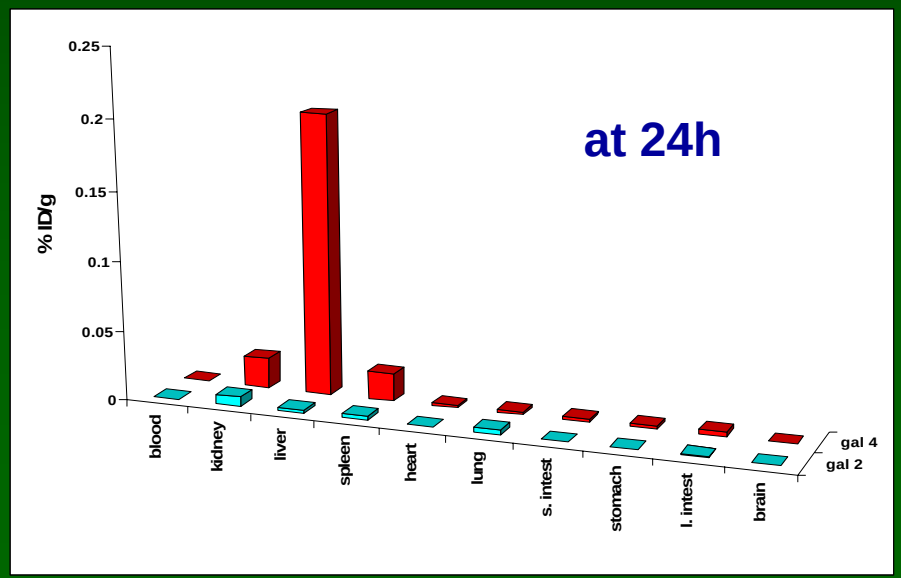
Biodistribution in Wistar rats

^{153}Sm(III)-glycoconjugates



- Gal conjugates target liver
  Gal < Gal2 ~ Gal4
- Lac conjugates intermediate
- Glc conjugates do not

Uptakes as % ID are small



- Gal conjugates target liver
  Gal < Gal2 < Gal4

Cluster effect

Conclusions

- A series of new DTPA glycosidase-resistant dendrimeric glycoconjugates was synthesized and chemically characterized
- The solution structure of some of their Ln complexes was studied by NMR
- The water relaxivity of their Gd(III) chelates was investigated by NMRD
- Preliminary studies of the pharmacokinetics and biodistribution of the ^{153}Sm-labeled glycoconjugates in Wistar rats shows selective liver uptake of the glycoconjugates with galactose terminal groups but not of the others
- These new glycoconjugate ligands may be selective to the liver ASGPR and are potentially useful for liver targeting and imaging ($^{153}Sm^{3+}$, $^{111}In^{3+}$ for gamma scintigraphy and Gd(III) for MRI)

Collaborations

-J. A. Martins, Paula Baía (Univ. Minho, Braga, PT)

- C. F. G. Geraldes (Dep. Biochemistry, Univ. Coimbra, PT)

- J.J P. Lima, I. Prata, A.C. Santos (Fac. Medicine, Univ. Coimbra, PT)

- M. Anjos Neves (ITN , Sacavém, PT)

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